Reg. No. GR/RNP/GOA/32

RNI No. GOAENG/2002/6410

Panaji, 21st June, 2007 (Jyaistha 31, 1929)

SERIES I No. 12

# OFFICIAL GAZETTE

## GOVERNMENT OF GOA

*Note : There are two Extraordinary issues to the Official Gazette, Series I No. 11 dated 14-06-2007 namely:–*

*(1) Extraordinary dated 14-6-2007 from pages 523 to 524 regarding Corrigendum from Department of Power (Office of the Chief Electrical Engineer).*

*(2) Extraordinary No. 2 dated 19-6-2007 from pages 525 to 526 regarding Notification from Department of Law & Judiciary (Legal Affairs Division).*

GOVERNMENT OF GOA

Department of Agriculture

Directorate of Agriculture

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**Notification**

3/5/EXT/M&F/19/2006-07/D.Agri

The Fertilizer (Control) Amendment Order, 2006 published by G.O.I. in the Gazette of India, Part II, Extraordinary No. 276, dated March 24, 2006, vide S. O. No. 391(E) dated 24-3-2006 is hereby published for general information of the public.

*S. S. P. Tendulkar,* Director of Agriculture.

Panaji, 14th July, 2006.

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MINISTRY OF AGRICULTURE

(Department of Agriculture and Co-operation)

**Order**

New Delhi, the 24th March, 2006

S. O. 391 (E).- In exercise of the powers conferred by section 3 of the Essential Commodities Act, 1955 (10 of 1995), the Central Government hereby makes the following Order further to amend the Fertilizer (Control) Order, 1985, namely:—

1. (1) This Order may be called the Fertilizer (Control) Amendment Order, 2006.

(2) It shall come into force on the date of its publication in the Official Gazette.

2. In the Fertilizer (Control) Order, 1985 (hereinafter referred to as the said Order), in clause 2 –

*(A)* after sub-clause *(a)*, the following sub-clause shall be inserted, namely:—

*"(aa)* Biofertilizer means the product containing carrier based (solid or liquid) living microorganisms which are agriculturally useful in terms of nitrogen fixation, phosphorus solublization or nutrient mobilization, to increase the productivity of the soil and/or crop;";

*(B)* in sub-clause *(h)*, for the words "and special mixture of fertilizers", the words ",special mixture of fertilizer, Bio-fertilizers Specified in Schedule III and Organic fertilizers Specified in Schedule IV" shall be substituted;

*(C)* after sub-clause *(o)*, the following sub-clause shall be inserted, namely:—

"*(oo)* Organic fertilizer means substances made up of one or more unprocessed material *(s)* of a biological nature (plant/animal) and may include unprocessed mineral materials that have been altered through microbiological decomposition process";

*(D)* in sub-clause *(q)* after the item iii, the following shall be inserted, namely:—

*(iv)* in relation to a Biofertilizer included in column 1 of Part A of Schedule III, the standard set out in the corresponding entry in column 2, subject to the limits of permissible variation as specified in part B of that Schedule;";

*(v)* in relation to a Organic Fertilizer included in column 1 of part A of Schedule IV, the standard set out in the corresponding entry in column 2, subject to the limits of permissible variation as specified in Part B of that Schedule."

3. In the said Order, for chapter heading "V. MANUFACTURE OF MIXTURE OF FERTILI-

ZERS", the chapter heading "V. MANUFACTURE OF MIXTURE OF FERTILIZERS, ORGANIC FERTILIZERS AND BIO-FERTILIZERS" shall be substituted.

4. In clause 12 of the said Order, for the words "or special mixture of fertilizer", the words "special mixture of fertilizer, Bio-fertilizer or Organic fertilizer" shall be substituted.

5. In clause 13 of the said Order, for sub-clause *(1)*, the following shall be substituted, namely:—

"*(1)* Subject to the other provisions of this Order,-

*(a)* no person shall manufacture any mixture of fertilizers whether of solid or liquid fertilizers specified in Part A of Schedule-I of the Order unless such mixture conforms to the standards set out in the notification to be issued by the state Government in the Official Gazette;

*(b)* no person shall manufacture any Bio-fertilizer unless such Bio-fertilizer conforms to the standards set out in the part A of Schedule-III;

*(c)* no person shall manufacture any Organic fertilizer unless such organic fertilizers conforms to the standards set out in the part A of Schedule-IV."

6. In clause 14, after sub-clause *(2)*, the following sub-clause shall be inserted, namely:—

"*(3)* Every person desiring to obtain a Certificate of Manufacture for preparation of Organic fertilizer or Bio-fertilizer shall make an application in Form D, in duplicate, together with a fee prescribed therefor under clause 36, to Registering authority".

7. In clause 15 of the said Order,—

*(i)* for the heading the following shall be substituted, namely:—

"Grant or refusal of Certificate of manufacture for preparation of mixture of fertilizers, Bio-fertilizers or Organic fertilizers" shall be substituted;

*(ii)* in sub-clause *(1)*, after the words "mixture of fertilizer" the words "Biofertilizer, Organic fertilizer" shall be inserted;

*(iii)* in sub-clause *(2)*, after the words "mixture of fertilizer" the words, "Bio-fertilizer or Organic fertilizers" shall be inserted.

8. In clause 17 of the said Order,—

*(i)* in the heading, after the words "mixture of fertilizer" the words ", Bio-fertilizer or Organic fertilizers" shall be inserted,—

*(ii)* after the words "mixture of fertilizers" the words ",Bio-fertilizer or Organic fertilizers" shall be inserted.

9. In clause 18 of the said Order,—

*(i)* in the heading, after the words "preparation of mixture of fertilizers", the words ",Bio-fertilizers or Organic fertilizer"shall be inserted;

*(ii)* in sub-clause *(1)*, after the words "mixture of fertilizer" the words "Biofertilizer or Organic fertilizer" shall be inserted.

10. In clause 21 of the said Order,—

*(i)* for the word "fertilizers", the words "fertilizers, Bio-fertilizers or Organic fertilizers" shall be inserted;

*(ii)* after sub-paragraph *(a)*, the following shall be inserted, namely:—

"*(aa)* Every container in which any Bio-fertilizer or Organic fertilizer is packed shall conspicuously be superscribed with the words BIO-FERTILIZERS/ORGANIC FERTILIZERS and shall bear only such particulars and unless otherwise required under any law nothing else, as may from time to time, be specified by the Controller in this behalf".

11. After clause 27A of the said Order, the following clause shall be inserted, namely:—

"27 B Qualifications for appointment of Inspectors for Biofertilizer and Organic Fertilizer no person shall be eligible for appointment as inspector of Bio-fertilizer and Organic Fertilizer under this Order unless he may possess the following qualifications, namely:-—

*(1)* Graduate in agriculture or science with chemistry/microbiology as one of the subjects; and

*(2)* training or experience in the field of quality control of Bio-fertilizers/Organic fertilizers.

12. In clause 28 of the said Order, in sub-clause 1, after entry *(b)* the following entries shall be inserted, namely:-

"*(ba)* draw samples of any Bio-fertilizers

in accordance with the procedure of drawal of samples laid down in Schedule III.";

"*(bb)* draw samples of any Organic fertili--zers in accordance with procedure of drawal of samples laid down in Schedule IV."

13. In clause 29 of the said Order, after sub-clause *(1)*, the following sub-clauses shall be inserted, namely,—

"*(1A)* Bio-fertilizer samples drawn by an inspector shall be analysed in accordance with the instructions laid down in Schedule III in the National Centre for Organic Farming, Ghaziabad or Regional Centers of Organic Farming at Bangalore, Bhubaneswar, Hissar, Imphal, Jabalpur and Nagpur or any other laboratory notified by Central or State Government.

"*(1B)* Organic fertilizer samples drawn by an inspector shall be analyzed in accordance with the instructions laid down in Schedule IV in the National Centre for Organic Farming, Ghaziabad or Regional Centers of Organic Farming at Bangalore, Bhubaneswar, Hissar, Imphal, Jabalpur and Nagpur or any other laboratory notified by Central or State Government."

14. In clause 30 of the said Order,-—

*(a)* in sub-clause *(1)*, after the word and letter "Form K", the following shall be inserted, namely, "and in case of Organic fertilizers and Bio-fertilizers in form K-l";

*(b)* in sub-clause *(2)*, after the word and letter "Form L" the following shall be inserted, namely,–

"and in case of Organic fertilizers and Bio-fertilizer in Form L 1."

15. in the said Order, after Schedule II, the following Schedules shall be inserted, namely,

**"Schedule III**

[*See* clause 2 *(h)* and *(q)*]

**PART - A**

**SPECIFICATION OF BIOFERTILISERS**

**1. Rhizobium**

| | | |
|---|---|---|
| (i) Base | = | Carrier based * or liquid based |
| (ii) Viable cell count | = | CFU minimum $10^7$ cell/g of carrier material or $10^7$ cell/ ml of liquid material. |
| (iii) Contamination level | = | No contamination at $10^5$ dilution |
| (iv) PH | = | 6.5-7.5 |
| (v) Particle size in case of carrier based material | = | All material shall pass through 0.15-0. 212 mm IS Sieve. |
| (vi) Moisture percent by weight, maximum in case of carrier based | = | 30-40% |
| (vii) Efficiency Character | = | Should show effective nodulation on all the species listed on the packet. |

*** Type of Carrier:**

**The carrier material such as peat, lignite, peat soil, humus, wood charcoal or similar material favoring growth of the organism.**

2. Azotobacter

| | | |
|---|---|---|
| (i) Base | = | Carrier based* or liquid based |
| (ii) Viable cell count | = | CFU minimum $10^7$ cell/g of carrier material or $10^7$ cell/ /ml of liquid material. |
| (iii) Contamination level | = | No contamination at $10^5$ dilution |
| (iv) pH | = | 6.5 — 7.5 |
| (v) Particle size in case of carrier based material | = | All material shall pass through 0.15- 0.212 mm IS Sieve |
| (vi) Moisture percent by weight, maximum | = | 30 — 40% |
| (vii) Efficiency Character | = | The strain should be capable of fixing at least 10 mg of nitrogen per g of sucrose consumed |

*** Type of Carrier:**

**The carrier material such as peat, lignite, peat soil, humus, wood charcoal or similar material favoring growth of the organism.**

**3. Azospirillum**

| | | |
|---|---|---|
| (i) Base | = | Carrier based* or liquid based |
| (ii) Viable cell count | = | CFU minimum $10^7$ cell/g of carrier material or $10^7$ cell/ /ml of liquid material. |
| (iii) Contamination level | = | No contamination at $10^5$ dilution |
| (iv) pH | = | 6.5 —7.5 |
| (v) Particle size in case of carrier based material | = | All material shall pass through 0.15 — 0.212 mm IS Sieve |
| (vi) Moisture percent by weight, maximum in case of carrier based | = | 30-40% |
| (vii) Efficiency Character | = | Formation of white pellicle in semisolid Nitrogen free bromothymol blue media. |

*** Type of Carrier:**

**The carrier material such as peat, lignite, peat soil, humus, wood charcoal or similar material favoring growth of the organism.**

**4. Phosphate Solubilising Bacteria**

| | | | |
|---|---|---|---|
| (i) | Base | = | Carrier based * or liquid based |
| (ii) | Viable cell count | = | CFU minimum $10^7$ cell/g of carrier material or $10^7$ cell/ml of liquid material. |
| (iii) | Contamination level | = | No contamination at $10^5$ dilution |
| (iv) | pH | = | 6.5 – 7.5 |
| (v) | Particle size in case of carrier based material | = | All material shall pass through 0.15–0.212 mm IS Sieve |
| (vi) | Moisture percent by weight, maximum in case of carrier based | = | 30–40% |
| (vii) | Efficiency Character | = | The strain should have phosphate solubilizing capacity in the range of minimum 30%, when tested spectrophoto metrically. In terms of zone formation, minimum 5 mm solubilization zone in prescribed media having at least 3 mm thickness |

*** Type of Carrier:**

**The carrier material such as peat, lignite, peat soil, humus, wood charcoal or similar material favoring growth of the organism.**

**Part - B**

**TOLERANCE LIMIT OF BIOFERTILISER**

5 X $10^5$ CFU/g of carrier or per ml of liquid material.

**Part - C**

**PROCEDURE FOR DRAWL OF SAMPLE OF BIO-FERTILISER**

**PROCEDURE FOR SAMPLING OF BIO-FERTILISERS**

**1. General Requirements**

1.0. In drawing, preparing and handling the samples, the following precautions and directions shall be observed.

1.1. Sampling shall be carried out by a trained and experienced person as it is essential that the sample should be representative of the lot to be examined.

1.2. Since the samples are also required for micro--biological analysis, utmost care is necessary to avoid extraneous contamination while drawing and handling the samples and to preserve them in their original conditions till they are ready for examination in the laboratory.

1.2.1 No preservatives or bactericidal/fungicidal agent shall be added to samples required for microbiological analysis.

1.3. Samples in their original un opened packets should be drawn and sent to the laboratory to prevent possible contamination of the samples during handling and help in revealing the true condition of the material.

1.4. Intact packets shall be drawn from a protected place not exposed to dampness, air, light, dust or soot and transferred to clean containers.

**2. Sampling Equipment**

2.1. A suitable scoop made of stainless steel may be used for drawing samples.

2.2. The sampling equipment shall be perfectly clean and sterile. It shall be properly sterilized by heating in a hot air oven at 160°C for not less than 2 h or by autoclaving for not less than 20 min at 120° C and held in suitable containers to prevent re-contamination.

**3. Scale of sampling**

3.1. Lot
All units (containers in a single consignment of type of material belonging to the same batch of manufacture) shall constitute a lot. If a consignment consists of different batches of the manufacture the containers of the same batch shall be separated and shall constitute a separate lot.

3.2. Batch
An inoculant prepared from a batch fermentor or a group of flasks (containers) constitute a batch.

3.3. For ascertaining conformity of the material to the requirements of the specification, samples shall be tested from each lot separately.

3.4. The number of packets to be selected from a lot shall depend on the size of the lot and these packets shall be selected at random and in order to ensure the randomness of selection.

**4. Drawal of samples :**

4.1. Three samples should be drawn separately from each lot.

**Part - D**

**METHODS OF ANALYSIS OF BIOFERTILISER**

**1. A. METHOD OF ANALYSIS OF RHIZOBIUM BIO-FERTILISERS**

**1. APPARATUS**

1.1. Pipettes Graduated 1 ml and 10 ml

1.2. Dilution Bottles or Flasks

1.3. Petri Dishes Clear, Uniform, flat- bottomed.

1.4 Hot-Air Oven
Capable of giving uniform and adequate temperature, equipped with a thermometer, calibrated to read up to 250° C and with vents suitably located to assure prompt and uniform heating.

1.5. Autoclave

1.6. Incubator

1.7. Hand Tally or Mechanical counting Device

1.8 pH meter

**2. REAGENTS**

2.1.Congo Red-one percent aqueous solution

2.2. Medium

Use a plating medium of the following composition:

| | |
|---|---|
| Agar | 20 g |
| Yeast Extract | 1 g |
| Mannitol | 10 g |
| Potassium hydrogen phosphate ($K_2 HPo_4$) | 0.5 g |
| Magnesium sulphate ($MgSO_4 7H_2O$) | 0.2 g |
| Sodium Chloride (NaCI) | 0.1 g |
| Congo red | 2.5 ml |
| Distilled water | 1000 ml |
| pH | 7.0 |

2.3. Sterilizing and preparation procedure for plates :

2.3.1. Sterilize the sampling and plating equipment with dry heat in a hot air oven at not less than 160°C for not less than 2 hours.

2.3.2. Sterilize the media by autoclaving at 120°C for 20 min. To permit passage of steam into and from closed containers when autoclaved, keep stoppers slightly loosened or plugged with cotton. Air from with in the chamber of the sterilizer should be ejected allowing steam pressure to rise.

Preparation Of Plating Medium And Pouring

2.3.3. Prepare growth medium in accordance with the composition of the specific biofertilisier.

2.3.4. Melt the required amount of medium in boiling water or by exposure to flowing steam in partially closed container but avoid prolonged exposure to unnecessarily high temperature during and after melting. Melt enough medium which will be used with in 3 h. Re--sterlisation of the medium may cause partial precipitation of ingredients.

2.3.5. When holding time is less than 30 min, promptly cool the molten medium to about 45°C, and store until used, in a water bath or incubator at 43 to 45°C. Introduce 12 to 15 ml of liquefied medium or appropriate quantity depending on size of the petridish at 42 to 44°C into each plate. Gently lift the cover of the dish just enough to pour in the medium, Sterlise the lips of the medium containers by exposure to flame.

a. immediately before pouring.

b. Periodically during pouring, and

c. When pouring is complete for each batch of plates, if portions of molten medium remain in containers and are to be used without subsequent sterilization for pouring additional plates. As each plate is poured thoroughly mix the medium with test portions in the Petri dish.

2.3.6. By rotating and tilting the dish and without splashing the medium over edge, spread the medium evenly over the bottom of the plate. Provide conditions so that the medium solidifies with reasonable promptness (5-10 min) before removing the plates from level surface.

**3. PREPARATION OF SERIAL DILUTIONS FOR PLATE COUNTS**

3.1 Dispense 30 g of Inoculant to 270 ml of sterile distilled demineralized water and shake for 10 min on a reciprocal shaker or homogeniser. Make serial dilutions up to $10^{10}$ Take 0.1 ml or suitable alliquots of $10^6$ to $10^9$ dilutions using sterile pipettes and deliver to Petri dishes containing set medium as given in 2.1 and spread it uniformly with a spreader. Invert the plates and promptly place them in the incubator.

**4. INCUBATION OF PLATES**

4.1. Label the plates and incubate at 28 + / -2°C for 3 to 5 days for fast growing Rhizobia and 5 to 10 days for slow-growing ones

4.2 Colony Counting aids

Count the colonies with the aid of magnifying lens under uniform and properly controlled, artificial illumination. Use a colony counter, equipped with a guide plate and rules in centimeter square. Record the total number of colonies with the hand tally. Avoid mistaking particles of undissolved medium or precipitated matter in plates for pin-point colonies. To distinguish colonies form dirt, specks and other foreign matter, examine doubtful objects carefully.

4.3. Count all plates but consider for the purpose of calculation plates showing more than 30 and less than 300 colonies per plate. Disregard colonies which absorb congo red and stand out as reddish colonies. Rhizobium stands out as white, translucent, glistening and elevated colonies. Count such colony numbers and calculate figures in terms of per litre, of carrier. Also check for freedom from contamination at $10^5$ dilution.

**5. TEST FOR NODULATION**

5.1 POT CULTURE TEST

**Plant Nutrient Solution**

| Composition | Concentration | g/1 |
|---|---|---|
| (a) Potassium chloride | 0.001 M | 0.0745 |
| (b) Potassium hydrogen Phosphate ($K_2HPO_4$) | 0.001 M | 0.175 |
| (c) Calcium sulphate ($CaS0_4\ 2H_2O$) | 0.002 M | 0.344 |
| (d) Magnesium sulphate ($MgS0_4\ 7H_2O$) | 0.001 M | 0.246 |
| (e) Trace elements solution: | | |
| (1) Copper sulphate ($CuS0_4\ 5H_2O$) | 0.01 mg/kg | 0.78 |
| (2) Zinc sulphate ($ZnS0_4\ 7H_2O$) | 0.25 mg/kg | 2.22 |
| (3) Manganese sulphate ($MnS0_4\ 4H_2O$) | 0.25 mg/kg | 2.03 |
| (4) Ammonium molybdate $[(NH_4)_6\ MO_7\ O_{24}.4H_2O]$ | 0.0025 mg/kg | 0.01 |
| (5) Boric acid ($H_3BO_4$) | 0.125mg/kg | 1.43 |

Prepare the solution No. (e) consisting of trace elements in one liter of stock solution and add final nutrient solution at the rate of 0.5 ml per litre.

| (f) Iron solution: | g/100ml |
|---|---|
| (1) ferrous sulphate | 5 |
| (2) Citric acid | 5 |

Prepare the solution No. (f) as 100 ml of stock solution and add final nutrient solution at the rate of 0.5 ml per litre.

**PREPARATION**

Prepare the nutrient solution by weighing out substances (a), (b) and (d) and dissolving them in a litre of water. To this solution add 0.5 ml of trace elements solution and 0.5 ml of iron solution. Grind in a mortar 0.344 g of calcium sulphate (c) to a fine consistency and add to the final nutrient solution. Autoclave the nutrient solution thus prepared at 120° C for 20 min.

**NOTES**

1. The nutrient solution may be prepared in the tap water provided the water is soft.
2. The nutrient solution should 4e shaken well to disperse calcium sulphate before dispensing.
3. If the solution is made up with distilled water, the pH is about 7.2 before autoclaving and falls to 5.5 on autoclaving and rises slowly on standing to about 5.8. However, there is no need to adjust pH. For most tropical legumes, pH of about 6.0 is adequate.

**5.3 PROCEDURE**

5.3.1. Immerse the seeds in 95 percent alcohol and follow by surface-sterilization in freshly prepared chlorine water (for 15 to 20 min) or 0.1 percent mercuric chloride solution 3 min in a suitable container such as a screw-capped biottle or a test-tube with a rubber hung. In case of seeds with tough seed coat, concentrated sulphuric acid may be used as a surface sterilant for 20 to 30 min. It is recommended that the seeds should be placed overnight in a desiccator containing calcium chloride before surface sterilization with sulphuric acid. Pour out the sterilant and wash the seeds in several changes of sterile water and wash the seeds in several changes of sterile water (at least ten times) to get rid of the sterilant. Fill earthenware or glazed pots with soil (2 parts soil and 1 part washed coarse sand) (pH 6 to 7) and autoclave for 2 h at 120°C. After two days incubation at room temperature, repeat autoclaving to ensure complete sterility of soil. Inoculate surface-sterilized seeds with a water slurry of the inoculant taken from a culture packet (15 to 100 g seeds per gram of inoculant depending on the size of the seed) and sow the seeds. Keep a set of pots with uninoculated seeds as control and also a set of pots with ammonium nitrate at the rate of 100kg N/ha as control aid incubate them in a pot-culture house during appropriate seasons for appropriate plants, taking care to separate the inoculated pots from the control pots. If growth rooms or cabinets having facilities to adjust temperature and light are available, the pots may be incubated in such controlled environmental conditions. Sterilize the nutrient solution at 120°C for 20 min and irrigate each pot once to the moisture holding capacity of soil. Subsequently, water the seedling periodically with sterilized water preferably through a plastic tube, taking care to prevent splashing of water from inoculated pots to uninoculated ones. Maintain required number of replicated pots (4 to 6) for each botanical species for statistical analysis.

5.3.2. After two to three weeks of growth, thin down the number of plants in each pot to four uniform plants. At the end of 6 to 8 weeks, take one set of pots from both the control and inoculated series and, separate the plants carefully from the soil under slow-running water. Obtain data on the number, colour (effective nodules are pink or red) and mass of nodules. At the end of 6 to 8 weeks, harvest the shoot system, dry at 60°C for 48 h and determine dry mass. For the above purpose, maintain adequate replications of pots (4 to 16).

5.3.3. Recorded the nodulation data regarding formation of pink colour of nodules as revealed visually when nodules are cut open by a razor blade. After computing the data, based on the dry mass of plants and nodulation data decide the effectiveness of cultures. If good effective pink modulation is obtained in inoculated plants together with local absence or sometimes presence of stray nodules in controls and if there is a 50 percent increase in the dry mass of plants over the uninoculated control without nitrate, it may be concluded that the culture is of the required quality.

**1. B. METHOD OF ANALYSIS OF AZOTOBACTER BIO-FERTILISER**

1. APPARATUS - same as of *Rhizobium*

2. REAGENTS :

2.1. Medium

Use a plating medium of the following composition

| | |
|---|---|
| Agar | 20 g |
| Sucrose ($C_{12}H_{22}O_{11}$) | 20.0 g |
| Ferric sulphate $Fe_2(SO_4)_3$ | 0.1 g |
| Dibasic potassium phosphate ($K_2HPO_4$) | 1.0 g |
| Magnesium sulphate ($MgS0_4, 7H_2O$) | 0.5 g |
| Sodium Chloride (NaCI) | 0.5 g |
| Calcium carbonate ($CaCO_2$) | 2.0 g |
| Sodium Molybdate ($Na_2MoO_4$) | 0.005 gms |
| Distilled water | 1000 ml |
| pH | 6.8 to 7.2 |

2.2. Sterilization & preparation procedure for plates: same as for Rhizobium

**PREPARATION OF PLATING MEDIUM AND POURING**

3. PREPARATION OF SERIAL DILUTIONS FOR PLATE COUNTS:

Dispense 30 g of Inoculant to 270 ml of sterile distilled water and shake for 10min on a reciprocal shaker. Make serials dilutions up to $10^{10}$ Take 0.1 ml or suitable allquots of $10^6$ to $10^9$ dilutions using sterile pipettes and deliver to Petri dishes containing set medium as given in 2.1 and spread it uniformly. Invert the plates and promptly place them in the incubator.

4. Incubation of plates :- Same as Rhizobium

4.1. Label the plates and incubate at 28+/-3°C for 4 to 6 days.

4.2 Colony counting aids: Same as Rhizobium

Azotobactor chrococcum colonies are gummy, raised with or without striations, viscous and open sticky. The pigmentation varies from very light brown to black. Count the colony number and observe the cyst formation as given below and calculate number per gram of the carrier material.

Grow the vegetative cells at 30°C on Burks agar medium comprising sucrose 20 g, dipotassium hydrogen phosphate 0.64 g, dihydrogen potassium phosphate 0.20 g; sodium chloride 0.20 g; calcium sulphate 0.05 g, sodium molybdate 0.001 g; ferric sulphate 0.003 g, agar 20 g and distilled water 1.000 ml. Look for vegetative cells after 18 to 24 h either by simple staining method or through a phase contrast microscope.

Grow the cyst cells on Burks agar medium as given above with 0.3 percent n-butanol in place of the carbon source. Look for cyst formation after 4 to 5 days in cubation.

5. TEST FOR NITROGEN FIXATION IN PURE CULTURE

5.1. PURE CULTURE MEDIUM

5.1.1. Prepare medium as given for Azotobacter (2.1 under 1 B), excluding agar.

5.2 PROCEDURE

Select from each *Azotobacter* colony, of the type that has been counted as *Azotobacter chroococcum* in. One colony and plate on the medium given in. Use this pure culture for inoculating the broth for nitrogen fixation. For this purpose, take 50-ml aliquots of broth in 250-ml conical flasks for inoculation. After 12 days growth at 28°C, test the contents of the flasks for purity by streaking on fresh medium and concentrating over a water-bath (50 to 60° C) to dryness. Wash the dried culture and take it as a sample. The contents of the flasks in inoculated control series should be processed in a similar manner.

5.3. Determination by Kjeldahl Method

(i) Reagents

(ii) Sulphuric acid-93-98 percent, N-free

(iii) Digestion mixture-Mix copper sulphate and potassium sulphate in the ratio 1: 10 and grind them to a fine powder.

(iv) Sodium hydroxide pellets or solution, N-free — For solution, dissolve about 450 g of sodium hydroxide in water, cool, and dilute to 1 litre (sp gr of the solution should be at least 1.36)

(v) Zinc granules - reagent grade.

(vi) Indicators :-

a) Methyl red indicator - Dissolve I g of methyl red in 200 ml of ethanol.

b) Mixed indicator - Prepare mixed indicator by dissolving 0.8 of methyl red and 0. 2 g of methyl blue in 500 ml of ethanol.

(vii) Hydrochloric or sulphuric acid - standard solution 0.5 or 0.1 N when amount of nitrogen is small.

(viii) Sodium hydroxide standard solution - 0.1 N (or other specified concentration).

*Note* - Ratio of salt to acid (m/v) should be about 1 : 1 at the end of the digestion for proper temperature control. Digestion may be incomplete at a lower ratio, and nitrogen may be lost at higher ratio. Each gram of fat consumes 10 ml of sulphuric acid and each gram of carbohydrate 4.0 ml of sulphuric acid during digestion.

5.4 Apparatus

(i) For digestion - Use Kjeldahl's flasks of hard, moderately thick, well-annealed glass with total capacity approximately 500 to 800 ml. conduct digestion over heating device adjusted to bring 250 ml of water at 25° C to rolling boil in about 5 minutes. To test the heaters, preheat for 10 minutes in the case of gas burners and for 30 minutes in the case of electric heaters. Add 3 to 4 boiling chips to prevent superheating.

(ii) For distillation - Use 500 – to 800-ml Kjeldahl's flask fitted with rubber stopper through which passes the lower end of an efficient scrubber bulb or trap to prevent mechanical carry-over of sodium hydroxide during distillation. Connect the upper end of the bulb tube to a condenser by a rubber tubing. Trap the outlet of the condenser in such a way as to ensure absorption of ammonia distilled over with the receiver.

(a) Procedure :-

Place 0.25 g of the sample in the digestion flask. Add 0.7 Gm mercuric oxide, 15 gm potassium sulphate followed by 25 ml of sulphuric acid. Shake, let stand for about 30 minutes and heat carefully until frothing ceases. Boil briskly until the solution clears and continue boiling further for 90 minutes. Cool, add about 200 ml of water, cool to room temperature and add a few zinc granules.

(b) Tilt the flask and carefully add 50 ml of sodium hydroxide solution without agitation. Immediately connect the flask to the distillation bulb on the condenser whose tip is immersed in 50 ml of standard 0.1 N acid in the receiving flasks. Rotate the digestion flask carefully to mix the contents. Heat until 150 ml of the distillate collects and titrate excess acid with 0.1 N base using methyl red or mixed indicator. Carry out blank determination on reagents.

*Note :* Check the ammonia recording periodically, using inorganic nitrogen control, for example, ammonium sulphate.

(c) Calculation :-

(i) Nitrogen content, percent by mass =

$$\frac{(\text{Milliliters of 0.1 N acid for sample - milliliters of 0.1 N acid for blank}) \times 0.14}{\text{mass of sample taken}}$$

(ii) Total nitrogen in culture = Total dry mass of sample x percent nitrogen.

(d) Take a 1.0 g of accurately weighed sample each from the inoculated series and from the controls. Put them separately in 250 ml volumetric flask, add 150 ml water, mix the content and make up the volume to 250 ml water. Shake for 5 minutes and centrifuge for 15 minutes at 10,000 rev/min. Estimate glucose in the supernatant in triplicate. The difference between the two provides the data of actual amount of glucose consumed. Calculate the amount of nitrogen fixed per gram of sucrose consumed.

5.5 Determination of Glucose :- From the supernatant, draw suitable aliquots and estimate reducing sugars (glucose) as follows.

(i) Reagents

(ii) Soxhlet modification of Fehling solution:- Prepare by mixing equal volumes of Solution A and Solution B immediately before using.

(iii) Copper sulphate solution (Solution A) - Dissolve 34.639 g of copper sulphate crystals ($CuSo_4 . 5H_2O$) in water, dilute to 500 ml and filter through glass wool or filter paper.

Standardization of copper sulphate solution:- Using separate pipettes, pipette accurately 5 ml of Solution A and 5 ml of Solution B into a conical flask of 250 ml capacity. Heat this mixture to boiling on an asbestos gauze and add standard invert sugar solution from a burette, about 1 ml less than the expected volume which will reduce the Fehling solution completely (about 48 ml). Add 1 ml of methylene blue indicator while keeping the solution boiling. Complete the titration within 3 minutes, the end point being indicated by change of colour from the blue to red. From the volume of invert sugar solution used, calculate the strength(s) of the copper sulphate solution by multiplying the titer value by 0.001 (mg/ml of the standard invert sugar solution). This would give the quantity of invert sugar required to reduce the copper in 5 ml of copper sulphate solution.

(iv) Potassium sodium tartrate (Rochelle salt) Solution (Solution B) :- Dissolve 173 g of potassium sodium tartrate and 50 g of sodium hydroxide in water, and dilute to 500 ml. Let the solution stand for a day, and filter.

(v) Hydrochloric acid-sp gr 1.18 at $20^0$C (approximately) 12 N)

(vi) Standard invert sugar solution - Weigh accurately 0.95 g of sucrose and dissolve it in 500 ml of water. Add 32 ml of concentrated hydrochloric acid, boil gently for 30 minutes and keep aside for 24 hours. Neutralize with sodium carbonate and make the final volume to 1000 ml; 50 ml of this solution contains 0.05 g of invert sugar.

(vii) Methylene blue indicator - 0.2 percent in water.

(viii) Procedure :- Place about 1 g (M), accurately weighed, of the prepared sample of AI into a 250 ml volumetric flask and dilute with about 150 ml of water. Mix thoroughly the contents of the flask and make the volume of 250 ml with water. Using separate pipettes, take accurately 5 ml each of Solution A and Solution B in a porcelain dish. Add about 12 ml of AI solution from a burette and heat to boiling over an asbestos gauze. Add 1 ml of methylene blue indicator and while keeping the solution boiling complete the titration within 3 minutes, the end point being indicated by change of colour from blue to red. Note the volume (H) in ml of AI solution required for the titration.

(ix) Calculation

Total reducing sugars, percent by mass = $\frac{250 \times 100 \times S}{H \times M}$

Where

S = strength of copper sulphate solution,
H = volume in ml of Al solution required for titration, and
M = mass in g of AI taken for the test.

5.6. Determination of Sucrose.

(i) Procedure:– To 100 ml of the stock AI solution, add 1 ml of concentrated hydrochloric acid and heat the solution to near boiling. Keep aside overnight. Neutralize this solution with sodium carbonate and determine the total reducing sugars as described in

(ii) Calculation

(a) Sucrose, percent by mass = (reducing sugars after inversion, percent by mass) - (reducing sugars before inversion, percent by mass) x 0.95.

(b) Nitrogen, mg per gram of sucrose consumed = 2 (a — b) — C
where

a = initial quantity of sucrose taken for the test,
b = mass of sucrose as calculated in (a), and
c = amount of nitrogen fixed per gram of glucose.

**1.C. METHOD OF ANALYSIS OF AZOSPIRILLUM BIO-FERTILISER**

1. APPARATUS : same as Rhizobium

**2. REAGENTS**

2.1. Medium

Use a plating medium of the following composition

| | |
|---|---|
| D-Malic acid | 5.0 g |
| Potassium hydroxide | 4.0 g |
| Di-potassium hydrogen phosphate | 0.5 g |
| Ferrous sulphate | 0.05 g |
| Manganese sulphate | 0.01 g |
| Magnesium sulphate | 0.1 g |
| Sodium chloride | 0.2 g |
| Calcium chloride | 0.1 g |
| Sodium molybdate | 0.002 g |
| Distilled water | 1000 ml |
| Boromothymol blue (0.5 % alcoholic solution) | 2.0 ml |
| Agar | 1.7 g |
| pH adjusted to 6.5 - 7.0 | |

2.2. Sterlising and preparation procedure for plates: same as Rhizobium

**PREPARATION OF PLATING MEDIUM AND POURING**

Same as Rhizobium

**3. PREPARATION OF SERIAL DILUTIONS FOR PLATE COUNTS :**

same as Rhizobium

**4. INCUBATION OF PLATES** :- Same as 4.1, p-26

4.1. Lable the plates and incubate at $36 \pm 1^0$C for 4 to 6 days.

4.2. Colony counting aids : Same as 4.2, p-26

**COUNTING**

Counting the tubes or plates which have turned blue in colour after inoculation and ascertain the presence of pellicles in undisturbed medium. To determine usual contamination on the same examine doubtful objects carefully.

Count all plates/tubes which have turned blue and consider them for the purpose of calculation. Count such type of tubes/plates and tally this count with MPN table Annex-E to get the number of cells per gram of the carrier.

Azospirillum Count/ /g of carrier = $\frac{\text{MPN table value x Dilution level}}{\text{Dry mass of product}}$

**1. D. METHOD OF ANALYSIS OF PHOSPHATE SOLUBULISING BACTERIAL BIO-FERTILISER**

1. APPARATUS : same as Rhizobium

**2. REAGENTS**

2.1. Medium

Use a plating medium of the following composition :

| | |
|---|---|
| Glucose | 10.0 g |
| Tri-calcium phosphate | 5.0 g |
| Ammonium sulphate | 0.5 g |
| Magnesium sulphate | 0.1 g |
| Sodium Chloride | 0.2 g |
| Yeast extract | 0.5 g |
| Manganese sulphate | Trace |
| Ferrous sulpahte | Trace |
| Distilled water | 1000 ml |
| Agar | 15.0 g |
| pH adjusted to 7 + / - 0.2 | |

2.2. Sterlising & preparation procedure for plates : same as Rhizobium

**PREPARATION OF PLATING MEDIUM AND POURING**
Same as Rhizobium

**3. PREPARATION OF SERIAL DILUTIONS FOR PLATE COUNTS :**
same as Rhizobium

**4. INCUBATION OF PLATES :-**
4.1. Label the plates and incubate at 28 + $1^0$C for 4 to 6 days.

4.2. Colony counting aids : Same as Rhizobium

**Counting**

Count the total number of colonies on the plates including colonies with solubilisation zone with the help of a colony counter.

Methods for counting solubilisation zones

a. Take 10g of PSBI (BF) in 90 ml in water
b. Make a ten fold dilution series up to $10^7$
c. Take 0.2 ml aliquote of $10^5$ to $10^7$ dilution using sterile pipettes and delivered to Petri dishes containing pikowskeyi media.
d. Spread it uniformly, Invert the plates and incubate them up to 2 weeks at 28+/-2°C.
e. Count the colonies showing hallow cones and measure their diameter. Minimum acceptable zone is 10 mm in diameter.

## 5. DETERMINATION OF SOLUBLE PHOSPHOROUS USING ASCORBIC ACID

5.1 APPARATUS

Spectrophotometer capable of transmission measurements at 840 to 880 nm.

Extractant: It is olsen extract.

5.2 REAGENTS

Ammonium Molybdate $[(NH_4)_6 MO_7O_{24}.4H_2O]$
L-Ascorbic Acid
p-Nitrophenol
$4NH_2SO_4$

5.3 PREPARATION OF REAGENTS

5.3.1. Sulphomolybdic Acid:-

-Take 20 g of ammonium molybdate and dissolve in 300 ml of distilled water.
-Add slowly 450 ml of 10 $NH_2SO_4$.
-Cool the above mixture and add l00 ml of 0.5 percent solution of antimony potassium tartrate.
-Coll and make the volume to one litre. Store in glass bottle away from direct sunlight.

5.3.2. Preparation of Mixed Reagent

Add 1.5 g of L-ascorbic acid in 100 ml of the above stock solution and mix. Add 5 ml of this solution to develop colour. Mixed reagent is to be prepared fresh as it does not keep for more than 24 h.

5.3.3. Procedure

(i) Weight the required material in a 100 ml conical flask.

(ii) Add 50 ml of extractant and shake it for 30 min. on a rotary shaker.

(iii) Filter the suspension through Whatman filter paper No. 40. If the filtrate is coloured then add a tea spoon of Dacro-60 (activated phosphorus free carbon), reshake and filter.

(iv) Take a known aliquot (5 to 25 ml) of the extract in a 50 ml volumetric flask.

(v) Add 5 drops of *p*-nitrophenol indicator (1.5 per cent solution in water) and adjust the pH of the extract between 2 and 3 with the help of $4NH_2SO_4$. The yellow colour will disappear when the pH of the solution becomes 3. Swirl gently to avoid loss of the solution along with the evolution of $CO_4$.

(vi) When the $CO_2$ evolution has subsided, wash down the neck of the flask and dilute the solution to about 40 ml.

(vii) Add 5 ml of the sulphomolybdic acid mixed reagent containing ascorbic acid, swirl the content and make up the volume.

(viii) Measure the transmission after 30 min at 880 nm using red filter. The blue colour developed remains stable up to 60 minutes.

(ix) Record the concentration of Phosphorus (P) in the extract from the standard curve and calculate the concentration of soluble Phosphorus as follows:

5.3.4. CALCULATIONS

(a) Weight of the substance taken = x g
(b) Volume of the extractant added = 50 ml
(c) Volume of the extract taken for P determination. = y ml
(d) Volume made after colour developed = 50 ml
(e) Reading from the standard curve against percent transmission recorded = z ppm
(f) Soluble Phosphorus, percent $P = \frac{z \times 50 \times l0^6 \times 50 \times l00}{y.x}$

5.3.5. Preparation of Standard Curve

Prepare standard curve using 0.1 to 0.6 ppm P in 50 ml volumetric flask. Plot the standard curve by taking concentration of soluble P on x-axis and percent T on y-axis using a semi-log graph paper. It is a straight line relationship between the soluble P and percent T when plotted on a semi-log graph paper.

## 6. MAINTENANCE AND PREPARATION OF CULTURE AND QUALITY CONTROL AT BROTH STAGE

**RHIZOBIUM:**

**1. Maintenance of pure cultures**

1.1. Maintain pure cultures of rhizobia on yeast extract mannitol agar (YEMA) slants of the following composition.

| | |
|---|---|
| Mannitol | 10.0 g |
| Potassium hydrogen phosphate ($K_2HP0_4$) | 0.5 g |
| Magnesium sulphate ($MgSO_4 7H_2O$) | 0.2 g |
| Sodium chloride (NaCl) | 0.1 g |
| Calcium carbonate ($Ca CO_3$) | 1.0 g |

| | |
|---|---|
| Yeast extract | 1.0 g |
| Agar | 18.0 g |
| Distilled water | 1 litre |
| pH | 6-8 - 7.0 |

1.2. Transfer a loopful of the pure culture to each of the agar slants aseptically in an inoculation room and incubate at 28 + /- 2° C for 3 to 10 days depending upon the species of Rhizobium. Always keep pure cultures at 4°C.

**2. Preparation of Inoculum Cultures**

2.1 Prepare yeast mannitol broth of the composition as given in 1.1. minus the agar.

2.2. Transfer a loopfull of the culture.

**3. Quality Control Tests Recommended at Broth Stage:**

3.1 Qualitative Tests
3.1.1. Check for freedom from visible contaminants
3.1.2. The pH of the bacterial broth shall normally be between 6.5 and 7.5
3.1.3. Smear and Gram stain
3.1. 3.1. Reagents

a. Ammonium oxalate crystal violet stain weigh 0.2 g of crystal violet and dissolve in 20 ml of 95 percent ethyl alcohol. Dissolve separately 0.8 g of ammonium oxalate in 80 ml of distilled water. Mix the two solutions and filter through a filter paper.

b. Iodine solution

| | |
|---|---|
| Iodine | 1.00 g |
| Potassium Iodide | 2.00 g |
| Distilled water | 300 ml |

Weigh the ingredients and dissolve in water. Filter through a filter paper.

c) Erythosine

| | |
|---|---|
| Erythrosine | 1.00 g |
| Phenol | 5.00 g |
| Distilled water | 100 ml |

Weigh the ingredients, dissolve in distilled water and filter through a filter paper.

3.1. 3. 2. Procedure

Prepare a smear on a clean microscope slide, fix over a flame by gentle and intermittent heating, air cool and flood with ammonium oxalate crystal violet stain for 1 min. After removing the excess of ammonium oxalate crystal violet, wash the slide under a gentle stream of running tap water. Flood the slide with iodine solution for half a minute remove excess stain wash with 95 percent ethyl alcohol and finally wash under a gentle stream of running tap water. Flood the slide with erythrosine stain for about 3 min, wash under a gentle stream of running tap water and dry between the folds of a filter paper. Examine the slide under a compound microscope using an oil immersion objective.

*Note* :- A smear prepared from undiluted broth should be free from Gram positive cells. The presence of a few gram positive cells in occasional fields which may be due to dead cells in the medium may be disregarded.

3.1.4. Absence of Growth on Glucose -Peptone Agar

The composition of the glucose - peptone agar is as follows :

| | |
|---|---|
| Glucose | 10.0 g |
| Peptone | 20.0 g |
| Sodium chloride (NaCI) | 5.0 |
| Agar (IS 6850) | 15.0 |
| Distilled water | 1000 ml |
| Bromocresol purple | 10 ml of 1.6 persons ethyl alcohol solution |
| pH | 7.2 |

*Note* :- When a loopful of the broth is streaked into this medium and incubated at 28 + / -2°C for 24 h, the purple-violet colour of the medium (due to the indicator bromocresol purple) shall not change. If the colour changes to yellow (acidic reaction) or blue (alkaline reaction) the broth is grossly contaminated. Hence, the broth should be rejected.

3.1.5. Streak on yeast Extract mannitol Agar with Congo Red

When a loopful of broth culture is streaked to a plate of this medium and incubated at 28 + /- 2° C for 3 to 10 days, it shall show colonies of bacteria with growth characteristics same as that of the pure culture used in the preparation of the broth, Other wise, the broth should be rejected.

3.2 Quantitative Test

3.2.1. Viable or Plate Counts

Serially dilute one milliliter of the broth to obtain dilutions of the order of $10^6$ to $10^9$. Plate 0.2 ml aliquots of the dilutions on YEMA plates and incubate at 28 + / - 2°C for 2 to 6 days, depending on the species of Rhizo- bium. The counts of viable Rhizobium in the final broth from shake culture or fermentors shall be not less than $10^8$ to $10^9$ cells/ml. Otherwise, the broth should be rejected.

**AZOSPIRILLUM**

1. Maintenance of pure cultures

1.1. Maintain pure culture of Azospirillum on nitrogen free bromothymol blue medium and maintain as semi solid medium

1.2. Transfer a loopful of pure culture to each of the agar

culture tube asceptically in an inoculation room and incubate 37 ±.2 °C for three days and keep it undisturbed.

Always keep pure culture below 5°C.

2. Preparation of Inoculum culture and Mass culture: Inoculum culture and mass culture of this standard shall be prepared as described for Rhizobium of this standard.

3. Quality Control Test Recommended at Broth Stage

3.1. Qualitative Test

3.1.1 Check for free from contaminants by preparing slide and observing under microscope.

3.1.2. The pH of becterial broth shall normally be between 7.0 to 8.0 .

3.1.3. Gram staining test shall be carried out as described for Rhizobium of this standard.

3.1.4. See the colour change in the media after 24 hours from inoculation. The colour will change from green to blue.

3.1.5. Watch the pellicle just below the surface of the media. It is checked on the third day after keeping inoculated broth undisturbed.

3.2. Quantitative Test

3.2.1. Most Probable Number (MPN) as given in Annexure-E. The counts of Azosprillium in the final broth from shake culture or fermentoes shall be not less than $10^8$ to $10^9$ cells/ml. Other wise the broth should be rejected.

**AZOTOBACTOR**

1. Maintenance of pure cultures.

1.1 Maintain pure cultures of Aztobactor on slants of the following composition.

| | |
|---|---|
| Agar | 20 gm |
| Sucrose | 20 gm |
| Ferrous Sulphate | 0.1 |
| Dibasic Potassium Phosphate | 1.0 gm |
| Magnesium Sulphate | 0.5 gm |
| Calcium carbonate | 2.0 gm |
| Sodium Molybdate | 0.005 gm |

1.2 Transfer a loopful the pure culture to each of agar slants aseptically in an inoculator room and incubate at 28+/- 2°C for 3 to 10 days depending upon the species of Azotabactor. Always keep culture pure cultures at 5°C

**2. Preparation of inoculum culture**

2.1 Prepare Jensens media broth of the compositor as given in 1.1 minus the agar

2.2 Transfer a loop full of the culture into a 100ml/ /250 m; conical flask containing the broth. Incubate the flasks at 28+/-2°C on a rotary shaker for 2 to 6 days.

3. Quality control Tests recommended by Broth stage.

3.1 Qualitative test.

3.1.1 Check for free from containments by preparing slide and observing under microscope.

3.1.2 The pH by bacterial broth shall normally be between 6.5 to 7.0

3.1.3. Gram staining test shall be carried out as described for Rhizobium of this standard.

3.2 Quantitative test.

3.2.1. Viable cell count same as Rhizobium

**4. Packing, Marking, Storage and use**

**4.1 PACKING**

Biofertiliser shall be packed in polyethylene packs, thickness which shall not be less than 75-100 micron.

**4.2 MARKING**

Each polyethylene pack shall be marked legibly and indelibly with the following information:

(a) Name of the product.
(b) Name and address of the manufacturer,
(c) Crop(s) for which intended;
(d) Type of the carrier used;
(e) Batch number;
(f) Date of manufacture;
**(g) Expiry date which shall not be more than 6 months from the date of manufacture;**
(h) Net mass in kg/gram and area meant for;
(i) Storage instruction worded as under;
"STORE IN COOL PLACE AWAY FROM DIRECT SUN LIGHT AND HEAT"
(*j*) Any other information required under the *standards of Weights and Measures (Packaged Commodities) Rule, 1977.*

**4.3** Items (c), (f) and (g) shall be printed on a coloured ink background.

**4.4** Direction for use of biofertiliser shall be printed briefly on the packets as given below.

"The contents of the packet are sufficient enough for seed treatment on to the given area to be broadcasted or given seedlings for root dipping depending on the specified crops as denoted on the packet. Mix the inoculants with seeds gently with the minimum amount of water, taking care to avoid damage to seed coat. Dry the inoculated seed under shade over clean surface gunny bag and sow them immediately.

Use only for the crops mentioned. Use before the expiry date and do not expose to direct sun light or heat.

Biofertiliser is not a chemical fertilizer hence to not mix inoculated seeds or inoculant with agro--chemicals."

**4.5 Storage**

Inoculant shall be stored by the manufacturer in a cool and dry place away from direct heat preferably at temperature of $20^0$C. It shall also be the duty of the manufacturer to instruct the retailers and, in turn, the users about the precautions to be taken during storage.

**SCHEDULE — IV**

[*See* clause 2(h) and (q)]

**Part - A**

**SPECIFICATION OF ORGANIC FERTILISER**

**1. City compost :**

| | | |
|---|---|---|
| (i) | Moisture, per cent by weight | 15.0-25.0 |
| (ii) | Colour | Dark brown to black |
| (iii) | Odour | Absence of foul odour |
| (iv) | Particle size | Minimum 90% material should pass through 4.0 mm IS Sieve |
| (v) | Bulk Density ($g/cm^3$) | 0.7-0.9 |
| (vi) | Total Organic Carbon, Per cent by weight, Minimum | 16.0 |
| (vii) | Total Nitrogen (as N) Per cent by weight, Minimum | 0.5 |
| (viii) | Total Phosphates (as $P_2O5$) Per cent by weight, Minimum | 0.5 |
| (ix) | Total Potash (as $K_2O$) Per cent by weight, Minimum | 1.0 |
| (x) | C:N ratio | 20: 1 or less |
| (xi) | pH | 6.5 -7.5 |
| (xii) | Conductivity (as $dsm^1$), Not more than | 4.0 |
| (xiii) | Pathogens | Nil |
| (xiv) | Heavy metal content (as mg/Kg) per cent by weight, Maximum | |
| | Arsenic (as $As_2O_3$) | 10.00 |
| | Cadmium (as Cd | 5.00 |
| | Chromium (as Cr) | 50.00 |
| | Copper (as Cu) | 300.00 |
| | Mercury (as Hg) | 0.15 |
| | Nickel (as Ni) | 50.00 |
| | Lead (as Pb) | 100.00 |
| | Zinc (as Zn) | 1000.00 |

**2. Vermicompost :**

| | | |
|---|---|---|
| (i) | Moisture, per cent by weight | 15.0-25.0 |
| (ii) | Colour | Dark brown to black |
| (iii) | Odour | Absence of foul odour |
| (iv) | Particle size | Minimum 90% material should pass through 4.0 mm IS Sieve |
| (v) | Bulk Density ($g/cm^3$) | 0.7- 0.9 |
| (vi) | Total Organic carbon per cent by weight, Minimum | 18.0 |
| (vii) | Total Nitrogen (as N) per cent by weight, Minimum | 1.0 |
| (viii) | Total Phosphate (as $P_2O$ 5) per cent by weight, Minimum | 1.0 |
| (ix) | Total Potassium (as $K_2O$) per cent by weight, Minimum | 1.0 |
| (x) | Heavy metal content (as mg/Kg) per cent by weight, Maximum | |
| | Arsenic (as $As_2O_3$) | 10.00 |
| | Cadmium (as Cd) | 5.00 |
| | Chromium (as Cr) | 50.00 |
| | Mercury (as Hg) | 0.15 |
| | Nickel (as Ni) | 50.00 |
| | Lead (as Pb) | 100.00 |

**3. Pressmud :**

| | | |
|---|---|---|
| (i) | Moisture, per cent by weight, Maximum | 15.0 |
| (ii) | Total Nitrogen (as N) per cent by weight, Minimum | 1.80 |
| (iii) | Total Phosphorous (as $P_2O_5$) per cent by weight, Minimum | 2.00 |
| (iv) | C :N ratio, Minimum | 10:1 |
| (v) | Total Potassium (as $K_2O$) per cent by weight, Minimum | 1.40 |
| (vi) | PH | 7.0-8.0 |
| (vii) | Heavy metal content (as mg/Kg) per cent by weight, Maximum | |
| | Arsenic (as $As_2O_3$) | 10.00 |
| | Cadmium (as Cd) | 5.00 |
| | Chromium (as Cr) | 50.00 |
| | Copper (as Cu) | 300.00 |
| | Mercury (as Hg) | 0.15 |
| | Nickel (as Ni) | 50.00 |
| | Lead (as Pb) | 100.00 |
| | Zinc (as Zn) | 1000.00 |

**Part - B**

**TOLERANCE LIMIT OF ORGANIC FERTILISER**

**0.1 unit for combined Nitrogen, Phosphorus and Potassium Nutrients**

**Part - C**

**PROCEDURE FOR DRAWL OF SAMPLE OF ORGANIC FERTILISER**

(As per methodology as mentioned under schedule-II, Part - A of FCO, 1985)

**The Inspector shall draw any sample of Organic Fertiliser in accordance with the procedure of drawal mentioned under Schedule-II, Part-A.**

**Part - D**

**METHODS OF ANALYSIS OF ORGANIC FERTILISER**

**1. Estimation of pH**

* Make 25 g of compost into a suspension in 50 ml of distilled water and shake on a rotary shaker for 2 hours.

* Filter through Whatman No.I or equivalent filter paper under vacuum using a Buchner funnel.
* Determine pH of the filtrate by pH mete.

**2. Estimation of moisture**

**Method :**

Weigh to the nearest mg about 5 gm of the prepared sample in a weighed clean, dry Petri Dish. Heat in an oven for about 5 hours at 65° +, -1°C to constant weigh. Cool in a desicator and weigh. Report percentage loss in weight as moisture content.

**Calculation :**

Moisture per cent by weight = $\frac{100(B-C)}{B-A}$

A = Weight of the Petri Dish
B = Weight of the Petri Dish plus material before drying
C = Weight of the Petri Dish plus material after drying

**3. Estimation of Bulk density**

**Requirement**

-100 ml Measuring cylinder Weighing balance
-Rubber pad [1 Sq foot; 1 inch Thickness] Hot air oven

**Method**

-Weigh a dry 100ml cylinder (WI gill)
-Cylinder is filled with the sample up to the 100 ml mark. Note the volume (VI ml)
-Weigh the cylinder along with the sample (W2 gm) -Tap the cylinder for two minutes.
-Measure the compact volume (V2 ml)
Calculation
Bulk density = $\frac{\text{Weight of the sample taken (W2-Wl)}}{\text{Volume (Vl-V2)}}$

**4. Estimation of EI.**

**Requirements**

-250 ml flak -Funnel [OD- 75 mm]
-100 ml Beaker -Analytical Balance
-Potassium Chloride [AR grade] -Filter paper
-Conductivity meter [With temperature compensation system]

**Method**

-Pass fresh sample of organic fertilizer through a 2-4 mm sieve.
-Take 20 gm of the sample and add 100 ml of distilled water to it to give a ratio of 1:5
-Stir for about an hour at regular intervals.
-Calibrate the conductivity meter by using O.OlM potassium chloride solution.
-Measure the conductivity of the unfiltered organic fertilizer suspension.

**Calculation**

Express the results as millimho's or ds/cm at 25°C specifying the dilution of the organic fertilizer suspension viz., 1:5 organic fertilizer suspension.

**5. Estimation of Organic Carbon**

**Apparatus :-**

(i) Conical flask 500 ml
(ii) Pipettes -2, 10 and 20 ml
(iii) Burette - 50 ml.

**Reagents:-**

(i) Phosphoric acid (Ortho) - 85%
(ii) Sodium Fluoride Solution - 2
(iii) Sulphuric Acid AR -96% containing 1. 25% Ag2S04
(iv) Standard IN K2Cr 207. Dissolve 49.04 g K2Cr207 in water and dilute to 1 litre.
(v) Standard 0.5 N Ferrous ammonium sulphate - Dissolve 196.1 g Fe. $(NH_4h(SO_4)_2 6H_2O$ in 800 ml water containing 20 ml conc. $H_2SO_4$ and dilute to 1 litre.
(vi) Diphenylamine indicator - Dissolve 0.5 g reagent grade diphenylamine in 20 ml water and 100 ml conc $H_2SO_4$

**Procedure:-**

Grind air dry compost and sieve with 80 mesh (2 mm sieve). Take 0.5 g sample in 500 ml conical flask. Add 50 ml of I (N) $K_2Cr_2O_7$ with burette and swirl a little. Then add 50 ml conc. $H_2SO_4$ and swirl again 2-3 times. Allow to stand for 30 minutes in a dark place and thereafter add 200 ml water. Then the volume was made upto 500 ml in a volumetric flask. Out of this 50 ml a aliquot (equivalent to 0.1 g sample) was taken in another 500 ml conical flask. Add 15 ml of orthophosphoric acid and 1 ml of Diphyneilamine indicator. Titrate with 0.5 N Ferrous Ammonium Sulphate till the colour flashes blue violet to green. Simultaneously run a blank without compost sample.

**Calculation :-**

$$\frac{10\,(BT)}{B} \times 0.003 \times \frac{100}{\text{Weight of sample}} \times 1.3$$

Organic Carbon % =

Where B = Volume (ml) of ferrous ammonium sulphate solution required for blank titration.
T = Volume (ml) of ferrous ammonium sulphate required for sample.

Source: Tandon, Methods of Soil, Plant, Fertilizer and Water Analysis p-16

**7. Estimation of total nitrogen**

As mentioned under Schedule-II, Part-B, 3 (v) of FCO (1985)

**8. Estimation of C : N ratio**

**Method**

Calculate the C:N ratio by dividing the organic carbon value with the total nitrogen value.

**9. Estimation of Phosphate**

As mentioned under Schedule-II, Part B, 4 (D) (ii) of FCO (1985)

*Note :* In the final aliquot for titration the concentration $P_2O_5$ should be 25-30 ml.

**10. Estimation of Potassium**

**Flame Photometry Method :-** Total potassium are usually determined by dry ashing at 650-700 Degree Centigrade and dissolving in concentrated hydrochloric acid.

**Reagent and Standard Curve**

(1) Potassium Chloride Standard Solution: Make a stock solution of l000 ppm K by dissolving 1.909 g. of AR Grade potassium chloride (dried at 60 Degree C. for 1 h) in distilled water 1; and diluting up to 1 liter. Prepare 100 ppm Standard by dididuting 100 ml of 1000 ppm stock solution to 1 litre with extracting solution.

(2) Standard cureve: Pipette 0, 5, 10, 15 and 20 ml of 100 ppm solution into 100 ml volumetric flasks and make up the volume upto the mark. The solutions contain 0, 5, 15 & 20 ppm K respectively.

**Procedure**

* Take 5g sample in a porceline crucible and ignite the materials to ash at 650-700 C. in a muffle furnace.

* Cool it and dissolve in 5 ml concentrated hydrochloric acid, transfer in a 250 ml beaker with several washing of distilled water and heat it. Again transfer it to a 100 ml volumetric flask and make up the volume.

* Filter the solution and dilute the filtrate with distilled water so that the concentration of K in the working solution remains in the range of 0 to 20 ppm, if required.

* Determine K by the flame photometer using the K-filter after necessary setting and calibration of the instruments .

* Read similarly the different concentration of K of the standard solution in flame photometer and prepare the standard curve by plotting the readings against the different concentration of the K.

**Calculation:** Potash (K) % by weight = R x 20 x diluting factor, where R = ppm of K in the sample solution (obtained by extra plotting from stand curve)

**11. Estimation of Heavy Metals**

**Arsenic :-** As mentioned under Schedule - II, Part B, 3 (xiv) of FCO (1985)

**Cadmium:-** As mentioned under Schedule - II, Part B, 8 (x) of FCO (1985)

**Copper:-** As mentioned under Schedule - II, Part B, 8 (iv) of FCO (1985)

**Lead:-** As mentioned under Schedule - II, Part B, 8 (v) of FCO (1985)

**Zinc :-** As mentioned under Schedule - II, Part B, 8 (ii) of FCO (1985)

**Chromium & Nickle**

**MATERIALS REQUIRED**

(a) Triacid mixture : Mix 10 parts of $HNO_3$(Nitric acid), 1 part $H_2SO_4$ (Sulphuric acid) and 4 parts of $HClO_4$ (Perchloric acid).
(b) Conical flask, 250 ml
(c) Hot Plate
(d) Whatman Filter paper No. 42
(e) Atomic Absorption Spectrophotometer (AAS)

**PROCESSING OF SAMPLE**

Take 5.0 g or suitable quantity of oven dried (105°C) sample thoroughly ground and sieved through 0.2 mm sieve in a conical flask.

Add 30 ml triacid mixture, cover it with a small glass funnel for refluxing. Digest the sample at 200°C on a hot plate till the volume is significantly reduced with a whitish residue.

After cooling, filter the sample with Whatman No. 42 filter paper, make up to 100 ml in a volumetric flask

**MEASUREMENT**

Estimate the metal concentrations of Cd, Cu, Cr, Fe, Pb, Ni, Zn using Atomic Absorption Spectrophotometer (AAS) as per the procedure given for instrument. Run a blank following the same procedure.

**EXPRESSION OF RESULTS**

Express the metal concentration as mg/g on oven dry weight basis in 3 decimal units (Reference: Manual for Analysis of Municipal Solid Waste (compost) -Central Pollution Control Board)

**Mercury**

**REAGENTS**

(a) Conc. Nitric acid ($HNO_3$)
(b) Conc. Sulphuric acid ($H_2SO_4$)
(c) Potassium persulphate (5% solution): Dissolve 50 g of $K_2S_20_8$ in 1 litre of distilled water.
(d) Potassium permagnate (5% solution) : Dissolve 50 g of $KMnO_4$ in 1 litre of distilled water.
(e) Hydroxylamine Sodium Chloride solution : Dissolve 120 g of Hydroxylamine salt and 120 g of Sodium Chloride (NaCl) in 1 litre distilled water.
(f) Stannous chloride (20%) : Dissolve 20 g of $SnCl_2$ in 100 ml distilled water.

**MATERIAL REQUIRED**

(a) Water Bath
(b) Flameless Atomic Absorption Spectrophotometer or Cold vapour Mercury analyzer.
(c) BOD bottle, 300 ml

**PROCESSING OF SAMPLE**

(a) Take 5 g (finely ground but not dried) sample in an oven at a temperature of 105° C for 8 hours for moisture estimation.

(b) Take another 5 g sample (finely ground but not dried) in a BOD bottle, add to it 2.5 ml of conc. $HNO_3$, 5ml of conc. $H_2SO_4$ and 15 ml of 5% $KMnO_4$

(c) After 15 minutes add 8 ml of 5% $K_2S_2O_8$ .

(d) Close the bottle with the lid and digest it on a water bath at 95°C, for 2 hours.

(e) After cooling to room temperature add 5 ml hydroxylamine sodium chloride soln.

MEASUREMENT

Reduction of the digested sample is brought out with 5 ml of 20% $SnCl_2$ immediately before taking the readings, using a cold vapour mercury analyzer

EXPRESSION OF RESULTS

Express the mercury concentration as mg/g on oven dry weight basis in 3 decimal units

(Reference : Manual for Analysis of Municipal Solid Waste (compost) - Central Pollution Control Board)

16. In the said Order, for Form D, the following form shall be substituted, namely :-

**"EMBLEM**
**FORM 'D'**

[ *See* clause 14 (2) and 18 (1) ]

**FORM OF APPLICATION TO OBTAIN A CERTIFICATE OF MANUFACTURE OF PHYSICAL/GRANULATED MIXTURE OF FERTILISER OF ORGANIC FERTILISER/BI0- FERTILISER**

To

The Registering Authority

Place, .....

State of.....

(1) Full Name and address of the applicant :

(2) Does the applicant possess the qualification prescribed by the State Government under sub-clause *(1)* of clause 14 of the Fertiliser control under, 1985.

(3) Is the applicant a new comer? (Say 'Yes' or 'No')

(4) Situation of the applicant's premises where physical/granulated mixture organic fertiliser/Bio-fertiliser will be prepared:

(5) Full particulars regarding specifications of the physical/granulated mixture of fertilisers/organic fertiliser/Bio-fertiliser for which the certificate is required and the raw materials used in making the mixture.

(6) Full particulars of any other certificate of manufacture, if any, issued by any other Registering Authority;

(7) How long has the applicant been carrying on the business of preparing physical/granulated mixture of fertilisers / organic fertiliser/Bio-fertiliser mixture of micronutrient fertilisers?

(8) Quantities of each physical/granulated mixture of fertilisers/mixture of micronutrient fertilisers/organic fertilisers/Bio-fertilisers (in tonnes) in my/ /our possession on the date of the application and held at different addresses noted against each;

(9) (i) If the applicant has been carrying on the business of preparing physical/granulated mixtures of fertilisers/mixture of micronutrient fertilisers/organic fertilisers/Bio-fertiliser, give all particulars of such mixtures handled, the period and the place (s) at which the mixing of fertilisers was done.

(ii) Also give the quantities of physical/granulated fertiliser mixture organic fertiliser/Bio-fertiliser handled during the past calendar year.

(10) If the application is for renewal, indicate briefly why the original certificate could not be acted on within the period of its validity .

**Declaration**

(a) I have deposited the prescribed registration certificate fee/renewal fee.

(b) I/We declare that the information given above is true and correct to the best of my/our knowledge and belief, and no part there is false.

(c) I/we have carefully read the terms and conditions of the certificate of manufacture given in Form F appended to the Fertiliser (control) order, 1985 and agree to abide by them.

(d) I/We declare that the physical/granulated mixture/organic fertiliser/Bio-fertiliser for which certificate of manufacture is applied for shall be prepared by me/us or by a person having such qualifications as may be prescribed by the State Government from time to time or by any other person under my/our direction, supervision and control or under the direction, supervision and control of person having the said qualification.

(e) I/We declare that the requisite laboratory facility specified by the Controller, under this Order is possessed by me/us.

*Name and address of applicant*
*in block letters:*

Date: *Signature of applicant (s)"*

Place:

17. In the said Order, for Form F, the following form shall be substituted, namely:-

**"FORM 'F'**

[*See* clause 15 (2) and 18 (2)]

Book No. ..... Certificate No. .....
Date of issue .....
Valid upto .....

**CERTIFICATE OF MANUFACTURE IN RESPECT OF PHYSICAL/GRANULATED MIXTURE/ORGANIC FERTILISER / BIO-FERTILISER**

..... (Name of Manufacturer) is hereby given the certificate for manufacture of the physical/granulated mixture/organic fertiliser/Bio-fertiliser specified below subject to the terms and conditions of this certificate and to the provisions of the Fertiliser (Control) Order, 1985.

| | |
|---|---|
| Full particulars of the organic fertiliser/Bio-fertiliser | Full address of the premises where the organic fertiliser/Bio-fertiliser will be made |
| Date: | Registering Authority: |
| Seal: | State: |
| | Renewed upto ..... |
| Date: | Registering Authority: |
| Seal : | State : |

Terms and conditions of this certificate

(1) The holder of this certificate shall display the original thereof in a conspicuous place open to the public in a part of the principal's premises in which business of making the physical/granulated mixture/organic fertiliser/bio-fertiliser is carried on and also a copy of such certificate in similar manner in every other premises in which that business is carried on. The required number of copies of the certificate shall be obtained on payment of the fees thereof.

(2) The holder of this certificate shall not keep in the premises in which he carries on the business of making physical/granulated mixture of biofertilisers/ /organic fertiliser in respect of which a certificate of registration has not been obtained under the fertilisers (Control) Order, 1985.

(3) The holder of this certificate shall comply with the provisions of the fertiliser (Control) Order, 1985 and the notification, order and direction, issued there under for the time being in force.

(4) The holder of the certificate shall report forth with to the Registering Authority any change in the premises specified in the certificate or any new premises in which he carried on the business of making physical/granulated mixture/organic fertiliser/biofertiliser and shall produce before the authority the original certificate and copies thereof so that necessary corrections may be made therein by that authority.

(5) The holder of this certificate shall ensure that the physical/granulated mixture/organic fertiliser/ /bio-fertiliser in respect of which a certificate of registration has been obtained is prepared by him or by a person having such qualifications, as may be prescribed by the State Government, from time to time or by any other person under the direction, supervision and control of the holder or the person having the said qualifications.

(6) The certificate and copies thereof, if any, will be machine numbered and delivered against the signature of the holder thereof or his agent on the carbon copy of the certificate which will be kept intact bound in the "Certificate Book" by each Registering Authority."

18. In the said Order, after form J, the following form shall be inserted, namely:-

**"FORM 'J-1'**

[*See* clause 28 (bb)]

**FORM INDICATING PARTICULARS OF ORGANIC FERTILISER/BIO-FERTILISERS SAMPLED**

(1) Name and address of dealer/manufacturer/importer .....

(lA) *Certificate of Registration Number.....

(2) Date of sampling .....

(3) Details of markings of bags from where sample has been taken .....
.....

(i)* Type organic fertiliser/of Bio-fertiliser .....
.....

(ii) Name of manufacturer/importer .....
.....

(iii) * Batch No. (if applicable) and date of manufacture/import .....

(iv) Composition .....

(4) Date of receipt of the stock by the dealer/manufacturer/importer/pool handling agency.....
.....

(5) *Code No. of sample .....

(6) Stock position of the lot .....

(7) Physical condition of organic fertiliser/Bio-fertiliser .....

(8) Whether samples drawn from open bags ** or

stiched bags **/with sealed packet .....
.....

(9) Name and Address of organic fertiliser/Bio-fertiliser Inspector drawing sample—
.....

* * For organic fertiliser

*Signature and Metallic Seal impression of Fertilizer Inspector*

**Receipt of the dealer**

Certified that the sample of organic fertiliser/ /Bio-fertiliser has been drawn in accordance with the procedure laid down in the fertiliser (Control) Order, 1985 from the stock in my possession, and I have signed the test samples at the time of wax sealing. I have also received one test sample out of the three test samples prepared.

*Signature and Seal of Fertiliser Inspector*

*Signature* of *dealer/manufacture/importer/pool handling agency with address"*

19. In the said Order, after Form K, the following form shall be inserted, namely :-

**"FORM 'K-1'**
(*See* clause 30)

**MEMORANDUM TO ACCOMPANY ORGANIC FERTILISER/BIO-FERTILISER SAMPLE FOR ANALYSIS**

No.

From

.....

.....

To,
In charge
Organic fertiliser/Bio-Fertiliser Quality Control Laboratory

The Bio-fertiliser samples as per details given below are sent for analysis:-

(1) *Name of organic fertiliser/Bio-fertiliser .....
.....

(2) Date of sampling.....

(3) Physical condition of organic fertiliser/Bio-fertitiser .....

(4) Code number of sample .....

2. The analysis report may be forwarded to .....
.....

Place:
Date:

*Signature and metallic seal impression of Fertilizer Inspector"*

20. In the said Order, after Form L, the following forms shall be inserted, namely:-

**"FORM 'L-l'**

(*See* clause 30)

**ANALYSIS REPORT OF ORGANIC FERTILISER SAMPLE**

No. .....

Government of . .....

.....

.....
*(Name of the Laboratory)*

Date.....

To
The Fertiliser Inspector
.....
.....

The analysis report of the organic fertiliser sample forwarded vide your reference No. .....
Dated .....is as per details given below:

(1) Name of Organic fertiliser .....
.....

(2) Date of Sampling.....

(3) Code No. of sample as indicated by the Inspector
.....

(4) Date of receipt of the sample in the Laboratory
.....

(5) Laboratory sample No. .....

(6) Date of analysis of sample .....

(7) Analysis of Organic Fertiliser (on fresh weight basis)

| Sl. No. | Specification as per/FCO | Composition as per analysis | Variation | Permissible Tolerance limit |
|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 |
| **(A) Physical Characteristics -** | | | | |
| | (i) Moisture content | ..... | | |
| | (ii) Bulk density | ..... | | |
| | (iii) Particle size | ..... | | |
| **(B) Chemical Characteristics -** | | | | |
| | (i) Total Organic Carbon | ..... | | |

(ii) Total Nitrogen .....
(iii) C:N .....
(iv) Phosphorus .....
(v) Potassium .....
(vi) pH .....
(vii) Conductivity .....

**(C) Heavy Metal**

(i) Cadmium .....
(ii) Chromium .....
(iii) Copper .....
(iv) Mercury .....
(v) Nickel .....
(vi) Lead .....
(vii) Zinc .....

Remarks : The sample is/is not according to specification and fails in .....
.....

Signature of the Incharge
(Testing Laboratory)

Copy to:-
Director of Agriculture
.....
.....

**FORM 'L - 2'**
*(See* clause 30)

**ANALYSIS REPORT OF BIO-FERTILISER SAMPLE**

No.....

Government of .....
.....
.....
(Name of the Laboratory)

Date .....

To

The Fertiliser Inspector
.....
.....

The analysis report of the organic fertiliser sample forwarded vide your reference No. .....
Dated ..... is as per details given below:

(1) Name of Biofertiliser .....
(2) Date of Sampling .....
(3) Code No. of sample as indicated by the Inspector .....
(4) Date of receipt of the sample in the Laboratory .....
(5) Laboratory sample No. .....
(6) Date of analysis of sample .....
.....

(7) Analysis of Bio-fertiliser (on fresh weight basis)

| Sl.No. | Specification as per FCO (Rhizobium, Azotobacter, Azospirillum, PSM) | Composition as per analysis (Rhizobium, Azotobacter, Azospirillum, PSM) | Variation | Permissible Tolerance limit |
|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 |
| | **(A) Physical Characteristics :-** | | | |
| | (i) Moisture content | | | |
| | (ii) Particle size | | | |
| | **(B) Chemical Characteristics :-** | | | |
| | (i) pH | | | |
| | **(C) Microbial Characteristics :-** | | | |
| | (i) Viable Cell Count | | | |
| | (ii) Contamination Level | | | |
| | **(D) Efficiency Characteristics:-** * | | | |
| | (i) Nodulation Test | | | |
| | **(ii) Nitrogen fixed (mg)/g of sucrose consumed | | | |
| | ***(iii) Formation of White pellicle in semi solid Nitrogen free bromothymol blue media | | | |
| | +(iv) (a) Solubilization zone (mm) b) P- phosphorus (%) Spectrophotometer. | | | |

*Rhizobium, * * Azotobacter, * * * Azospirillum, + PSM

Remarks : The sample is/is not according to specification and fails in .....
.....

Signature of the Incharge
(Testing Laboratory) "

Copy to:-
Director of Agriculture
.....

[No. 9-23/2005-Org. Fing.]
SATISH CHANDER, Jt. Secy.

*Note :-* The Fertilizer (Control) Order, 1985 was published in the Gazette of India, vide number G. S. R. 758 (E) dated the 25th September, 1985 and subsequently amended vide number:-

1. G. S. R. 201(E) dated 14th February, 1986
2. G. S. R. 508 (E) dated 19th March, 1986
3. G. S. R. 1160 (E) dated 21st October, 1986
4. S. O. 822 (E) dated 14th September, 1987

5. S. O. 1079 (E) dated 11th December, 1987
6. S. O. 252 (E) dated 11th March, 1988
7. S.O. 724 (E) dated 28th July, 1988
8. S.O. 725 (E) dated 28th July, 1988
9. S.O. 940 (E) dated 11th October, 1988
10. S.O. 498 (E) dated 29th June, 1989
11. S.O. 581 (E) dated 27th July, 1989
12. S.O. 673 (E) dated 25th August, 1989
13. S. I. 738 (E) dated 15th September, 1989
14. S.O. 140 (E) dated 12th February, 1990
15. S.O. 271 (E) dated 29th March, 1990
16. S.O. 403 (E) dated 23rd May, 1990
17. S.O. 675 (E) dated 31st August,1990
18. S.O. 261 (E) dated 16th April, 1991
19. S.O. 444 (E) dated 2nd July, 1991
20. S.O. 530 (E) dated 16th August, 1991
21. S.O. 795 (E) dated 22nd November, 1991
22. S.O. 377 (E) dated 29th May, 1992
23. S .O. 534 (E) dated 20th July, 1992
24. S. O. 826 (E) dated 9th November, 1992
25. S. O. 154 (E) dated 3rd June, 1993
26. S. O. 397 (E) dated 18th June, 1993
27. S. O. 942 (E) dated 10th December, 1993
28. S. O. 163 (E) dated 14th February, 1994
29. S. O. 340 (E) dated 17th April, 1995
30. S. O. 459 (E) dated 22nd May, 1995
31. S. O. 835 (E) dated 12th October, 1995
32. S. O. 575 (E) dated 20th August, 1996
33. S. O. 57 (E) dated 22nd January, 1997
34. S. O. 329 (E) dated 12th May, 1999
35. S. O. 1068(E) dated 4th November, 1999
36. S. O. 49 (E) dated 16th January, 2003
37. S. O. 373 (E) dated 1st April, 2003
38. S. O. 413 (E) dated 7th April, 2003
39. S. O. 540 (E) dated 4th May, 2003
40. S. O. 342 (E) dated 18th March, 2005.

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## Department of Official Language

### Directorate of Official Language

—

**Order**

2/5/2007/DOL/Dir./105

Ex post facto sanction of the Secretary (Official Language) is hereby accorded for continuation of temporary post of Director of Official Language created by Personnel Department vide Order No. 5/9/2000-PER dated 28-8-2003 and transferred to the Directorate of Official Language vide Order No. 5/9/2003-PER dated 28-6-2004 for further period of one year from 29-08-2006 in pay scale of Rs. 10000-325-15200.

Salaries of the Director shall be debitable to the Budget Head : 2070 - Other Administrative Services, 00-, 119 - Official Language, 01- Department of Official Language (Non Plan), 01 - Salaries.

*Prasad V. Lolayekar,* Director of Official Language & ex officio Joint Secretary.

Panaji, 11th June, 2007.

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## Department of Sports & Youth Affairs

### Directorate of Sports & Youth Affairs

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**Order**

DSYA/ADM/AD/PF-CREATION/2004

Sanction of the Government is hereby accorded for creation of two posts of Assistant Director of Physical Education, Goa General Service Group 'B' Gazetted, in the pay scale of Rs. 6500-200-10500.

The expenditure on the above shall be debited to the Budget Head as detailed below:-

2204 - Sports & Youth Services
101 - Physical Education
04 - Directorate of Sports (NP)
01 - Salaries

This ssue with the concurrence of the Finance (Revenue & Control) Department vide their U. O. No. 534 - F dated 19-02-07 and ARD's approval vide their Note No. DS/ADM/AD/PF-CREATION/2004, Adm. Reform Department dated 27-12-2006.

By order and in the name of the Governor of Goa.

Dr. *Susana de Sousa,* Director of Sports & Youth Affairs/ex officio Joint Secretary.

Panaji, 12th June, 2007.

www.goagovt.nic.in/gazette.htm
GOVERNMENT PRINTING PRESS,
PANAJI - GOA.
Price : Rs. 20.00